Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Association of intron and exon polymorphisms of *p53* gene in Iranian patients with gastritis

Rouhallah Najjar Sadeghi[1], Negar Sahba[1], Mohsen Vahedi[2], Seyed Reza Mohebbi[1], Mohammad Reza zali[1]

[1] *Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2] *Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ABSTRACT

Aim: The propose of this study was to evaluate the probable correlation between exon and intron polymorphisms of *p53* gene and their association with clinicopathological aspects of gastritis.

Background: regarding to the decisive role of *p53* in the development of a variety of human cancers, a comprehensive study concerning probable correlation between polymorphisms in the p53 intron and exon in gastritis lesions, may open new insight toward gastric cancer development and prevention.

Patients and methods: PCR-Sequencing was done for exons and introns 2-7 on the 97 gastritis and normal samples, age range of 15-83 years. Also, microsatellite status was evaluated using five mono nucleotide repeat markers. Variation at codon 72 was associated with IVS2+38, p53INS3 and IVS3-29. In addition, IVS2+38 had association with polymorphism at codon 36 & 245. Gastritis samples had stable microsatellite except nine patients showing polymorphism for NR-21 and one for Bat-25

Results: Most of patients with stable microsatellites (83.9%) had allele G at codon72 without p53INS3. In addition, all patients with GA and CG at codon 36 / IVS2+38 had stable microsatellites.

Severity and activity of gastritis were in association with genotypes combined of codon 36/IVS2+38 and 245/IVS2+38 respectively. In addition, the profiles of combined variation at codon 72/IVS3-29 and codon 72/IVS6+31 were different between patients with ages less and greater than 45 years.

Conclusion: As, some exon variations of p53 gene specially codon 72, were in association with alterations at introns and their combined genotypes were correlated with microsatellite status, pathological findings and age, therefore, it could be inferred that the these combinations of *p53* gene polymorphisms work as a whole, not as single.

Keywords: Gastritis, *p53* gene, *Helicobacter pylori*, Codon 72, IVS2+38.

(Please cite as: **Najjar Sadeghi R, Sahba N, Vahedi M, Mohebbi SR, zali MR. Association of intron and exon polymorphisms of *p53* gene in Iranian patients with gastritis. Gastroenterol Hepatol Bed Bench 2013;6(Suppl 1):S45-S51).**

Introduction

p53 is an important protein in human biology. One of the major roles of p53 is that it regulates the cell cycle integrity (1) therefore, functions as a tumor suppressor. *p53* prevents aberrant cell proliferation following diverse cellular stresses such as hypoxia, DNA damages, radiation, or chemotherapeutic drugs (2, 3) through regulation of expression of a number genes (4, 5), thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. For this reason, *p53* has been named as "the guardian of

Received: 28 April 2013 *Accepted*: 18 July 2013
Reprint or Correspondence: Rouhallah Najjar Sadeghi, PhD student. Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Iran
E-mail: najjarsadeghi@yahoo.com

Table 1. Details of primers for PCR amplification for exons and introns of p53 gene.

	Primers	Coverage*
Exon and intron 2&3	Forward:5' TCTCAGACACTGGCATGGTG 3' Reverse: 5' GGCAAGGGGGACTGTAGATG 3'	12853-13319
Exon and intron 4	Forward: 5'CTAGCAGAGACCTGTGGGAAG 3' Reverse: 5' CACTGACAGGAAGCCAAAGG 3'	13115-13702
Exon 5 & 6 and intron 4, 5, 6	Forward: 5' TTGTTTCTTTGCTGCCGTC 3' Reverse: 5' CCCCCTACTGCTCACCTGG 3'	14259-14799
Exon 7 and intron 6, 7	Forward: 5'GCGACAGAGCGAGATTCC 3' Reverse: 5'CTGAGTGGGAGCAGTAAGGAG 3'	15181-15588

*Coverage column shows the site of primers attachment according to gene bank data (Accession number AY838896.1)

the genome" because of its role in conserving stability by preventing genome mutation.

Since its discovery in 1979, *p53* has been the focus of attention of scientist throughout the world. According to some reports, more than 50% of adult human tumors bear inactivating mutations or deletions in the *p53* gene (6) which reflects its importance as a tumor suppressor gene. Therefore, disruption of its function(s) has a salient effect on the integrity of cell cycle and confers a selective advantage for tumor cells progression.

p53 gene abnormalities appear to be key factors in the development of gastric cancer and can be observed at early stages of carcinogenesis such as gastritis and maybe subsequent genetic abnormalities enable these precancerous lesions to grow toward neoplasm and ultimately gastric cancer (7, 8). Also, these alterations can be used as a biomarker for early diagnosis and to determine prognosis of intermediate stages or even for risk assessment of cancer development.

There is limited information on the *p53* alterations in gastric cancer and its precancerous lesions from Iranian population(9, 10) while this population experiences one of the highest rate of incidence and mortality for gastric cancer in the world (11). In addition, most of previous studies on gastritis were only limited to *p53* exon polymorphisms, and there is little work on the introns(12, 13), while the sequence environ SNPs can modulate its impact on the function and structure of the resultant protein. In line with this claim, the hypothesis that the genetic variation flanking codon 72 could affect *p53* function and characteristics is not new.

It is conceivable that some residues be collectively involved in the special functions of *p53*, not a single residue, for example, residues from 66-326 have a critical role in the *p53* degradation by E6 (14). Also, there is some contrary regarding to which allele of codon 72 is a susceptibility marker for gastric cancer (15), maybe these conflicting findings results from considering this codon itself or with different neighboring sequences in different studies. Therefore, evaluation of some polymorphisms together and determining their probable interaction may delineate some discrepancies.

There is no information about the probable correlation of exon and intron polymorphisms of *p53* especially in gastric cancer and its precancerous lesions. Therefore, it seems valuable to do a comprehensive study on the probable correlation between exon and intron polymorphisms of *p53* gene and their association with clinicopathological aspects.

Cells lacking *p53* exhibit a condition of genomic instability (16, 17). Therefore, *p53* function is necessary for genomic stability. Apparently *p53* has direct or indirect roles in the recognition of DNA damages and preserves genomic stability by regulating repair systems (18,

19) or cell death (20).Therefore, examination of a marker for genomic stability status such as microsatellite status will be valuable. Microsatellite instability (MSI) is a genome-wide alteration characterized by some global instability of repetitive microsatellite sequences (21). Hence, we decided to evaluate the probable correlation between exon and intron polymorphisms of *p53* gene and their correlation with MSI status to find additional insight about their potential effects on gastritis development and probable interplay among these processes during gastritis genesis.

Patients and Methods

This study was approved by the ethics and scientific committee of *Gastroenterology and Liver Diseases Research Center*, Shahid Beheshti university of Medical Sciences. The patients were informed about the aims of this study and were empowered to participate in this study. During endoscopic examination, samples from gastritis lesion and normal endoscopic appearance were taken from the patients. The histological grading was performed according to the update Sydney classification. *H.pylori* (HP) infection was evaluated histologically. None of samples was immunohistochemically positive for *p53* protein (9).

DNA extraction

The DNA from gastric biopsies was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR amplification and mutation analysis using direct sequencing

To search for nucleotide alterations of the *p53* gene the primers were design to cover exon 2-7 and some intron region especially donor and acceptor splice sites. PCR-sequencing was done using primers (table 1,) as follows:

PCR was carried out in 25 µl of reaction containing 1x PCR buffer, 1.5 mM $MgCl_2$, 10 pmol of each primer, 200 µM of each dNTP and half a unit Taq polymerase. The PCR conditions for each region were as follow: initial cycle of 5 min at 94°C and then 30 cycles of 30s at 94°C, 30s at 62.1°C for exon 2&3, 59.5 °C for exon 4, 63°C for exon 5&6, 62.1°C for exon 7, 45s at 72°C that was concluded by 10 min at 72°C. DNA Sequencing was done using ABI3130*xl* Genetic Analyzer.

Microsatellite instability analysis

Fragment analyzing (ABI 3130*xl* Genetic Analyzer, Applied Biosystems, Bedford, MA, USA) was done using five microsatellite markers: NR-27, NR-21, NR-24, BAT-25 and BAT-26. The samples were classified as high MSI-high if two or more markers showed instability, or MSI-low when only one marker demonstrated instability (22).

Immunohistochemical analysis of mismatch repair proteins

Immunohistochemical staining for MLH1, MSH2 and MSH6 proteins was done as previously described method (23). Patients with at least one abnormal above factors were considered in abnormal MMRP group.

Statistical analysis

SPSS13 software (chi^2 test and ANOVA) was used to evaluate the association of *p53* nucleotide alterations and clinicopathological findings, MSI and IHC. For all tests the significance level was set at 5%.

Results

Histologically confirmed 97 samples of gastritis lesion were included in the study, 41 male (42.3%, mean age: 44.5 ±17.7) and 56 female (57.7%, mean age: 42.6 ±15.6) in the age range of 15-83 years (43.41±16.48) (Table 2).

Nucleotide changes were seen in exon and intron regions as follows: codons 9, 36, 47, 72, 146, 213, 244, 245, 248, 257, IVS2+38 C>G

(rs1642785), IVS3+40-41ins16 (*p53*INS3: rs17878362), IVS3-29C>A (rs17883323), IVS6+31A>G (rs34949160), IVS7+72C>T (rs12947788), IVS7+92T>G (rs12951053).

Table 2. Pathological grading of gastritis lesions according to the update Sydney classification

Pathology	N	Mean age	Standard Deviation
Moderate active	33	41.5758	15.25211
Moderate	39	42.0769	17.33324
Severe active	21	45.5714	16.05482
Severe	4	60.2500	14.77329
Total	97	43.4124	16.48342

There were some associations between intron and exon polymorphisms: codon 72 and IVS2+38 (p <0.001), codon 72 and *p53*INS3 (p <0.001), codon 72 and IVS3-29 (p 0.028), codon 72 and IVS6+31 (p 0.05) (Table 3), codon 36 and IVS2+38 (p 0.015), codon 245 and IVS2+38 (p <0.001), codon 146 and IVS7+72 (p 0.043) (Table 4).

In the next level, we analyzed the correlation between these associated SNPs with clinicopathological aspects (see the next sections). For details of exon and intron alterations and their correlation with clinicopathological aspect please see next references (9, 12).

Gastritis samples had stable microsatellites, but nine patients showing polymorphism with variant allele for NR-21 and one for Bat-25.

Most of patients with stable microsatellites (83.9%) had allele G (CG or GG) at codon72 without p53INS3 while the other patients had NR-21 polymorphism (p 0.013). In addition, all patients with GA and CG at codon 36 / IVS2+38 had stable microsatellites (p= 0.01).

According to specific staining for HP, 87.6% of samples were HP positive. HP infection had no correlation with combined polymorphisms.

There was statistical significant correlation between pathology findings and variation at some polymorphic sites. Most patients (66.7%) with GA/CG and all patients with GA/ CC at codon 36 / IVS2+38 had severe gastritis lesion (p 0.022).

Also, most patients (60.4%) with GG/ CG at codon 245/IVS2+38 were pathologically active, while most patients (54.5%) with GG / GG were not active (p= 0.034).

There was statistical association between categorized age and polymorphism at codon 72 / IVS3-29 (p 0.017) and codon 72 / IVS6 +31 (p 0.016), So 50% of patients with age less than 45 years have GG and CC at codon 72 / IVS3-29 while 62.2 % of patients with age greater than 45 had CG and CC.

In addition, 50% of patients with age less than 45 years had GG and AA genotypes at codon 72 / IVS6 +31 while 64.4 % of patients with age more than 45 years had CG and AA genotype (p 0.016). No association was seen between any combination of intron and exon polymorphisms with gender.

Table 3. Statistical correlation between codon 72 and intron variations of p53 gene in gastritis lesion. The number in parentheses shows percentages.

		IVS2+38			IVS3-29		IVS6+31		Insertion		
		CC	**CG**	**GG**	**CC**	**CA**	**AA**	**AG**	**Homo**	**No**	**Hetro**
Codon 72	**CC**	8(57.1)	5(35.7)	1(7.1)	11(78.6)	3(21.4)	13(92.9)	1(7.1)	5(35.7)	4(28.6)	5(35.7)
	CG	0	45(95.7)	2(4.3)	43(91.5)	4(8.5)	47(100)	0	1(2.1)	27(57.4)	19(40.4)
	GG	0	4(11.1)	32(88.9)	36(100)	0	36(100)	0	1(2.8)	32(88.9)	3(8.3)
P value		< 0.001			0.028		0.05		< 0.001		

Table 4. Correlation between some exon and intron polymorphisms of p53 gene in gastritis lesion

		Codon 36	
		GG	GA
IVS2+38	CC	6(6.5)	2(40)
	CG	51(55.4)	3(60)
	GG	35(38.1)	0
	P value	0.015	
		Codon 245	
		GG	GT
IVS2+38	CC	5(5.7)	3(60)
	CG	50(56.8)	1(20)
	GG	33(37.5)	1(20)
	P value	< 0.001	
		Codon 146	
		GG	GC
IVS7+72	CC	77(88.5)	4(57.1)
	CT	9(10.5)	3(42.9)
	P value	0.043	

Discussion

Gastritis is a precancerous lesion of gastric cancer (24). According to some reports, about 7300 new cases in Iran (10.5 per 100,000 individuals) are afflicted to gastric cancer annually (11) with the 5-year survival rate of 23.6% and the median life expectancy of 19.9 months (25). Therefore, it seems necessary to evaluate and determine molecular events which originate from non-cancerous lesions and lead to malignancy.

Sequential changes in the gastric mucosa may occur over a period of years as a result of exposure to a variety of exogenous and/or endogenous factors which cause genetic alterations such as *p53* mutations and/or select an allele at polymorphic sites. Therefore, early detection of *p53* alterations at the level of precancerous lesions such as gastritis may provide new windows useful for the identifying patients prone to gastric cancer and prevention from gastric cancer development.

According to our findings, variation at codon 72 was statistically associated with *p53*INS3, IVS2+38 and IVS3-29.The codon 72 and *p53*IN3 have been extensively studied as putative susceptibility variants in some cancers with inconsistent results. *p53*IN3 is the only intronic polymorphism, that has been associated with an increased risk of several types of cancer (26). On the other hand, there is some inconsistency about the codon 72 as susceptibility allele for cancer. One possibility is that codon 72 is not the susceptibility allele itself, but instead marks a haplotype with the true susceptibility allele, or that codon 72 is the susceptibility factor and its surrounding SNP (such as *p53*IN3 or other intron/exon SNPs) mark it. The proximity of *p53*INS3 to the codon 72 polymorphism in exon 4 might partly explain the proposed association of this allele with some cancer.

Regarding our findings, it is not surprising to claim that these associated alterations work together during gastritis development.

If either multiple alleles affect susceptibility or codon 72 is the only marker for the actual susceptibility factor is not clear, therefore, it deserves to do a comprehensive study on the multiple SNPs in the different ethnic backgrounds to elucidate this issue. Previous work on gastric cancer and other neoplasia focus on the alterations in the exon regions. Therefore, there is no information about the probable importance and effects of these associated alterations on *p53* functions.

Our findings about the association between some combination of polymorphisms and MSI support the notion of correlation between *p53* and microsatellite status and indicate that some polymorphisms of *p53* gene are correlated with genomic instability. These polymorphisms may affect genomic stability at the nucleotide level through the effect of resultant p53 protein on the repair systems. This is supported by the observation that *p53* is capable of attaching to insertion/deletion loops, the DNA lesions associated with MSI (27). On the other hands, these variations may lead to the down/ up regulation of MMR system (28). In this study, we found no MMR defects in relation to *p53*

genotypes and microsatellite status, but as we follow MMR system through qualitative IHC, therefore, a quantitative study is recommended to quantify MMR expression.

Regarding current findings concerning to association between the alterations and pathological aspects, it seems possible that GG and CG at codon 245 &IVS2+38 has a protective effect against the activity. According to previous work (9, 12), these intron and exon alterations alone had no association with activity. Regarding the severity of gastritis most patients with GA/CG and GA/ CC at codon 36 / IVS2+38 had severe gastritis while the patients with other combination of these polymorphisms were diagnosed as moderate gastritis. These findings imply the idea that some functions of a protein and the resultant phenotypes depend on the specific combination of its polymorphisms and also polymorphisms work as a whole not as single "Many hands make light work".

CG / CC genotypes at codon 72 / IVS3-29 and CG / AA genotype at codon72 / IVS6+31, were seen predominantly at age greater than 45 years. Therefore, it can be concluded that these combined genotypes have a protective effect against gastritis development while GG / CC and GG / AA genotypes at these sites disposes one to develop gastritis and maybe gastric cancer at the ages less than 45 years, an issue which needs more study.

To our knowledge this is the first comprehensive study on gastritis lesion from view of the size of study and molecular processes which were evaluated such as *p53* exon and intron 2-7, MSI and Immunohistochemistry. In conclusion, the results of this study were shown that some clinicopathological aspects of gastritis lesion are correlated to the co-occurrence of some polymorphisms of *p53* gene. Among them, the combination of codon 72 and IVS2+38 with some intron and exon variations had some utmost importance. More studies are needed to delineate the probable molecular events leading to these observations.

Acknowledgements

This work was supported by Gastroenterology and Liver Diseases Research Center, Shahid Beheshti university of Medical Sciences, Tehran, Iran. The authors would like to thank unsparing helps of our lab personnel.

References

1. Mirzayans R, Andrais B, Scott A,Murray D. New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. J Biomed Biotechnol 2012; 2012:170325.

2. Ling Bai,Wei-Guo Zhu. p53:Structure, Function and Therapeutic Applications. J Cancer Mol 2006; 2:141-53.

3. Carstens M, Krempler A, Triplett A, Van Lohuizen M,Wagner K. Cell Cycle Arrest and Cell Death Are Controlled by p53-dependent and p53-independent Mechanisms in Tsg101-deficient Cells. J Biol Chem 2004; 279:35984-994

4. Mirza A, Wu Q, Wang L, McClanahan T, Bishop W R, Gheyas F, et al. Global transcriptional program of p53 target genes during the process of apoptosis and cell cycle progression. Oncogene 2003; 22:3645-54.

5. Menendez D, Inga A,Resnick M A. The expanding universe of p53 targets. Nat Rev Cancer 2009; 9:724-37.

6. Berkson R, Hollick J, Westwood N, Woods J, Lane D,Lain S. Pilot screening programme for small molecule activators of p53. Int J Cancer. 2005; 115:701-10.

7. Ochiai A, Yamauchi Y,Hirohashi S. p53 mutations in the non-neoplastic mucosa of the human stomach showing intestinal metaplasia. Int J Cancer 1996; 69:28-33.

8. Romiti A, Moretti A, Vecchione A, Muraro R, Feudi M, Rinaldi V, et al. Analysis of p53 expression in precancerous and malignant gastric mucosa. Oncol Rep 1998; 5:109-13.

9. Najjar Sadeghi R, Azimzadeh P, Vahedi M, Mirsattari D, Molaei M, Mohebbi S R, et al. Profile and frequency of p53 gene alterations in gastritis lesions from Iran. Digestion 2011; 83:65-75.

10. Najjar Sadeghi R, Vahedi M, Zojaji H,Zali M R. Correlation between global genome methylation and mutation at CpG codons of p53 gene. J Dig Dis 2013; 14:305-10.

11. Mehrabian A, Esna-Ashari F, Zham H, Hadizadeh M, Bohlooli M, Khayamzadeh M, et al. Gastric Cancer Prevalence, According To Survival Data in Iran. Iran J Public Health 2010; 39:I27-31.

12. Najjar Sadeghi R, Damavand B, Vahedi M, Mohebbi S R, Zojazi H, Molaei M, et al. Detection of p53 Common Intron Polymorphisms in Patients with Gastritis Lesions from Iran. Asian Pac J Cancer Prev 2013; 14:91-96.

13. Najjar Sadeghi R, Azimzadeh P, Vahedi M, Molaei M, Mohebbi S R, Zojaji H, et al. 16-bp insertion polymorphism of p53 gene in gastritis lesion. Gastroenterol Hepatol Bed Bench 2010; 3:115-19.

14. LI X, Coffino P. High-Risk Human Papillomavirus E6 Protein Has Two Distinct Binding Sites within p53, of Which Only One Determines Degradation. J Virol 1996; 70: 4509–16.

15. Zhou Y, Li N, Zhuang W, Liu G J, Wu T X, Yao X, et al. P53 codon 72 polymorphism and gastric cancer: A meta analysis of the literature. Int J Cancer. 2007; 121:1481-86.

16. Milinkovic V, Bankovic J, Rakic M, Milosevic N, Stankovic T, Jokovic M, et al. Genomic instability and p53 alterations in patients with malignant glioma. Exp Mol Pathol 2012; 93:200-206.

17. Hanel W, Moll U M. Links between mutant p53 and genomic instability. J Cell Biochem 2012; 113:433-39.

18. Smith M L,Seo Y R. p53 regulation of DNA excision repair pathways. Mutagenesis 2002; 17:149-56.

19. Ford J M. Regulation of DNA damage recognition and nucleotide excision repair: Another role for p53. Mutat Res 2005; 577:195-202.

20. Pietsch E C, Sykes S M, McMahon S B, Murphy M E. The p53 family and programmed cell death. Oncogene 2008; 27:6507-21.

21. Imai K,Yamamoto H. Carcinogenesis and microsatellite instability: The interrelationship between genetics and epigenetics. Carcinogenesis 2008; 29:673-80.

22. Buhard O, Suraweera N, Lectard A, Duval A,Hamelin R. Quasimonomorphic mononucleotide repeats for high-level microsatellite instability analysis. Dis Markers 2004; 20:251-57.

23. Molaei M, Mansoori B K, Ghiasi S, Khatami F, Attarian H, Zali M. Colorectal cancer in Iran: Immunohistochemical profiles of four mismatch repair proteins. Int J Colorectal Dis 2010; 25:63-69.

24. Papavassilliou E, Savva S. Gastritis and Gastric Cancer: Time for gastric cancer prevention. Ann Gastroenterol 2009; 21:218-22.

25. Zeraati H, Mahmoudi M, Kazemnejad A, Mohammed K. Postoperative life expectancy in gastric cancer patients and its associated factors. Saudi Med J 2005; 26:1203-07.

26. Whibley C, Pharoah P, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer 2009; 9:95-107.

27. Lee S, Elenbaas B, Levine A, Griffith J. p53 and its 14 kDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell 1995; 81:1013-20.

28. Xinarianos G, Liloglou T, Prime W, Sourvinos G, Karachristos A, Gosney J R, et al. p53 status correlates with the differential expression of the DNA mismatch repair protein MSH2 in non small cell lung carcinoma. Int J Cancer 2002; 101:248-52.